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Journal
Brain research. Developmental brain research, 77(1)

ISSN
0165-3806

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Publication Date
1994

DOI
10.1016/0165-3806(94)90220-8

Published in final edited form as:
Brain Res Dev Brain Res. 1994 January 14; 77(1): 133–136.

Status epilepticus results in reversible neuronal injury in infant rat hippocampus: novel use of a marker

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Abstract

Despite ready induction of severe limbic status epilepticus by systemic kainic acid (KA) in infant rats, excitotoxic neuronal injury has not been observed. The mechanisms of this resistance of the immature hippocampus to excitotoxicity are unknown. Acid fuchsin stain has been used as a marker of irreversibly injured neurons in the adult brain. We speculated that the dye might map reversibly injured neurons in the infant. Subsequent to KA-induced status epilepticus in 11-day-old rats, acid fuchsin stain was evident in the hippocampal CA3, CA1, dentate gyrus and hilus by 24 h, peaked at 48 h and disappeared by 6 days, without evidence for neuronal loss. Acid fuchsin may be a useful tool for delineating the distribution of reversibly injured immature neurons in experimental seizure paradigms.

Keywords

Excitotoxicity; Acid-fuchsin; Cell death; Seizure; Neonate; Kainic acid; Hippocampus

In the adult, kainic acid-induced status epilepticus (KA-SE) results in pyramidal neuron death in the hippocampal CA3, CA1 and hilar regions[7,10]. The immature hippocampus is remarkably resistant to seizure-induced, excitotoxic neuronal loss[12,16,17]. In view of the enhanced excitability of hippocampal neurons during the second and third postnatal week in the rat[19], and the severe behavioral seizures induced by KA, the nature of ‘protective’ mechanisms preventing excitotoxic death in these neurons has been a focus of investigation[5,13,15,21].

Acid fuchsin (AF), a biological stain[6], has been utilized extensively in studies of hypoglycemic[2], traumatic[9] and excitotoxic neuronal death[14,21]. In time-course studies, using serial sections, it has been established that the dye delineates ‘moribund’ neurons, which have suffered irreversible injury and disappear within a week[2].

We investigated the utility of AF as a marker of reversibly injured neurons subsequent to KA-SE in the infant brain. We studied the time-course and pattern of AF staining and of neuronal loss in hippocampal regions in 11- to 12-day-old rats in comparison to adults.

Experimental animals and paradigm

Sprague–Dawley-derived infant rats (11- to 12-day-old) of both sexes, were obtained and housed as published[3]. Adult males (225–250 g) were used for comparison. SE was induced by the maximal tolerated KA dose for each age (mortality 10–15%) 1.2–1.5 mg/kg

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intraperitoneally for infants, and 18 mg/kg subcutaneously for adults[1,8,13,20]. These KA doses resulted in severe SE for several hours[1,20]. In pilot studies, the presence of sustained epileptiform discharges in infant rats was verified via bipolar depth electrodes positioned in the dorsal hippocampus[3].

Tissue processing

Rats (n = 3 per time-point) were sacrificed at 1, 4, 12, 24, 48, 96 and 144 h. Adults survived 24 or 96 h. Rats were perfused under deep pentobarbital anesthesia with saline, followed by 4% phosphate-buffered paraformaldehyde. Brains were removed immediately, fixed overnight, and cryoprotected with 25% sucrose. Forebrain sections (20 μm) were mounted on gelatin-coated slides and kept at room temperature. One-in-five sections were processed for AF, and adjacent ones for hematoxylin and eosin (H&E).

Acid-fuchsin protocol

Sections were dipped sequentially in 4% buffered paraformaldehyde solution for 20 min, phosphate-buffered saline for 10 min, and distilled water for 2 min. Slides were then placed in a solution of 10 mg/ml AF (Sigma, St. Louis, MO) in 0.1% glacial-acetic acid for 20–30 s, depending on the freshness of the solution. Sections were rinsed in distilled water for 2 min, then dehydrated in 100% ethanol for 2 min and cover-slipped.

The time-course and intensity of AF stain in hippocampal CA1, CA3, dentate gyrus (DG) and hilus of infant rats are shown in Table I. Representative photomicrographs of AF-stained sections at 4 h (not different than controls) and 48 h are seen in Fig. 1a,b. A higher magnification of the hilus region at 48 h post KA-SE (Fig. 1c), reveals intense AF staining of both hilar and DG neurons. Fig. 2 demonstrates the resolution of AF staining by the sixth day in the infant hippocampus (Fig. 2a), with no evidence of pyramidal cell loss in CA3 (Fig. 2b, arrow), on H&E staining. An H&E-stained section from an adult hippocampus reveals significant loss of both CA3 and hilar neurons (Fig. 2c, arrows).

Limbic KA-SE, results in selective hippocampal cell loss and synaptic reorganization in adult but not in infant rats[7,10–12,16–18]. The cascade of molecular events leading from membrane depolarization to neuronal death has been shown to differ in these two age groups[4,5,13,15,17,21]. Irreversible injury in mature neurons may involve protracted up-regulation of immediate early genes[15], which is not found in the infant rat[13]. Auer et al. demonstrated that adult brain regions with a majority of AF-staining neurons subsequent to hypoglycemic injury, were depleted of neurons by 7 days[2]. AF staining may thus signify severe, irreversible cellular injury in these neurons[2,9,14]

The targets of AF, a tri-sulfonated triphenyl-methane, remain obscure[2,6]. Decreased cellular pH, as expected with metabolic failure, enhances the stain, but AF fully decolorizes only in a highly basic environment (pH 12–14[6]). A more plausible alternative explanation for its affinity to irreversibly injured neurons[2] is enhanced cell penetration due to increased membrane permeability, coupled with irreversible binding to denatured proteins[1,6]. Our results suggest that in immature neurons, injury sufficient to provoke AF staining does not necessarily result in neuronal death. Thus, AF may be a useful tool for ‘mapping’ neurons subjected to cellular stress or injury in the infant hippocampus, where other such cellular markers may not be informative[4,13].

In conclusion, selected hippocampal neurons in both infant and adult hippocampus are stained with AF subsequent to status epilepticus. While the stain signifies irreversible injury leading to cell death in the adult, it may provide a marker for reversible neuronal injury in

the immature hippocampus. AF may thus be useful in the study of the consequences of SE in the immature brain.

Acknowledgments

Supported by NIH NINDS NS28912, NS01307 (T.Z.B.), and student awards from Childrens Hospital Los Angeles and USC (D.C). We thank Prof. J. Motte for helpful discussions.

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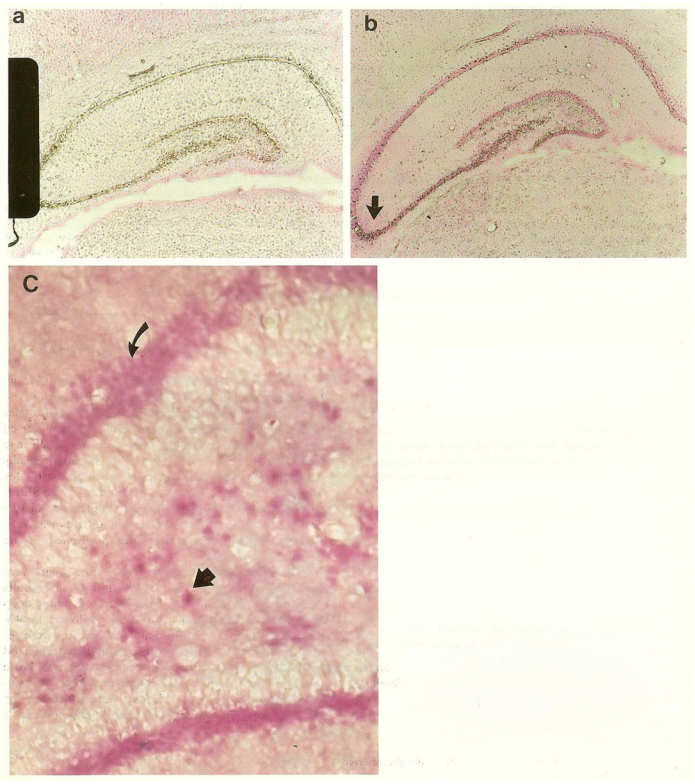

Fig 1.

Photomicrographs of acid-fuchsin (AF) staining of 11-day-old rats subsequent to kainic-acid-induced status epilepticus (KA-SE). a: AF stain, 4 h from onset of SE. Bar = 1.8 mm. b: AF stain in a rat surviving for 48 h; bright pink neurons are visible in CA3 (arrow), CA1, dentate gyrus and hilus. c: high magnification of AF stain at 48 h; bright pink hilar (arrow) and DG neurons (curved arrow). 300 ×.

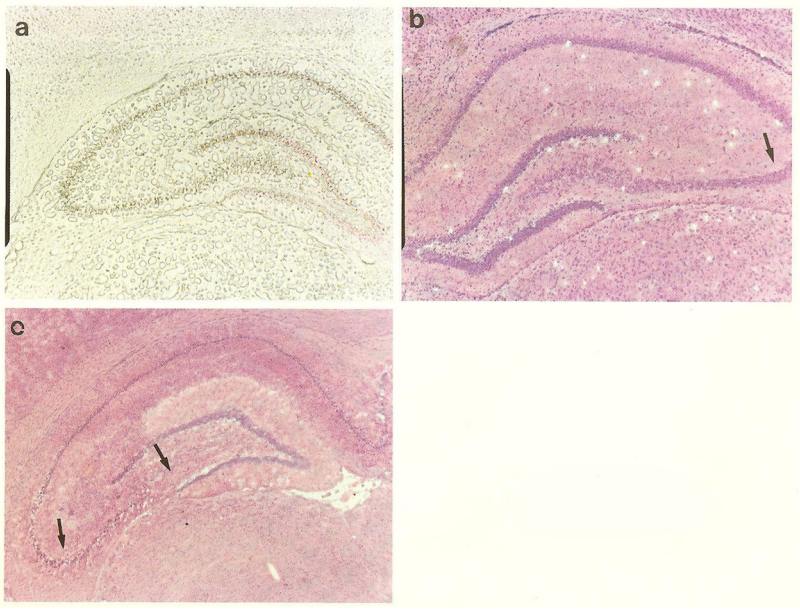

Fig 2.

Photomicrographs of 11-day-old (a,b) and adult (c) hippocampi 4–6 days subsequent to KA-SE. a: resolution of AF stain by 144 h. Bar = 1.6 mm. b: H&E stain demonstrates the integrity of CA3 (arrow), and presence of hilar neurons. c: H&E-stained adult hippocampus 96 h post KA-SE shows neuronal loss in CA3 (arrows) and hilar subfields.

TABLE I

Time-course of status-epilepticus-induced AF stain in infant hippocampus

Time (h)	CA1	CA3	Dentate gyrus	Hilus
4	−	−	+/−	+/−
12	+/−	−	+/−	+/−
24	+ + +	+ +	+ + +	+ +
48	+ + +	+ + +	+ +	+ +
96	+	+/−	+ +	+
144	−	−	+/−	−